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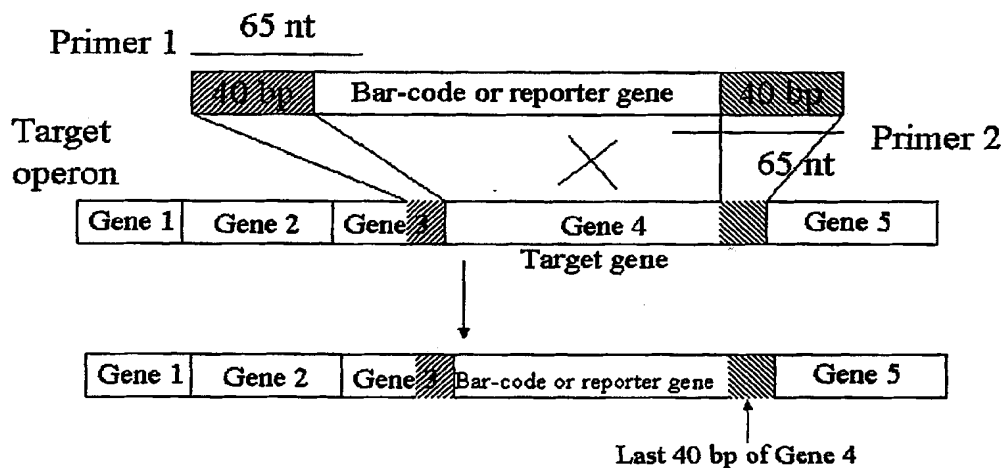
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- Published:
— with international search report
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

[Continued on next page]

(54) Title: METHODS FOR GENE DISRUPTION AND USES THEREOF

Strategy for Gene Disruption



(57) Abstract: The present invention relates to compositions and methods for in-frame disruption of a gene sequence by homologous recombination. The present invention may be used in certain embodiments to disrupt a gene without causing any downstream effects on non-target sequences. In certain embodiments, the inventive methods may be used to identify and/or characterize products encoded by essential genes, conditionally essential genes, and non-essential genes.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

In national Application No
PCT/CA 02/01160A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/09 C12N15/65 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| X | LINK A J ET AL: "METHODS FOR GENERATING PRECISE DELETIONS AND INSERTIONS IN THE GENOME OF WILD-TYPE ESCHERICHIA COLI: APPLICATION TO OPEN READING FRAME CHARACTERIZATION" JOURNAL OF BACTERIOLOGY, WASHINGTON, DC, US, vol. 179, no. 20, October 1997 (1997-10), pages 6228-6237, XP000890089 ISSN: 0021-9193 abstract page 6229, column 1, paragraph 1 page 6229, column 2, paragraph 7 -page 6230, column 2; figure 1 page 6231, column 1, paragraph 3 -page 6234, column 1, paragraph 1 page 6235, column 1, paragraph 3 -page 6236, column 1, paragraph 3; figures 2-6 --- -/-- | 1-29 |

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the international search

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19/09/2003

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 02/01160

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
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| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | WO 00 44906 A (ELITRA PHARMACEUTICALS INC) 3 August 2000 (2000-08-03) page 13, line 28 -page 15, line 18; example 5 page 16, line 15 - line 29 | 1-29 |
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| A | LORENZ M C ET AL: "Gene disruption with PCR products in <i>Saccharomyces cerevisiae</i> " GENE, ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, NL, vol. 158, no. 1, 1995, pages 113-117, XP004206649 ISSN: 0378-1119 the whole document | 1-29 |
| A | ZAMBROWICZ B P ET AL: "Disruption and sequence identification of 2,000 genes in mouse embryonic stem cells" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 392, 9 April 1998 (1998-04-09), pages 608-611, XP002111633 ISSN: 0028-0836 the whole document | 1-29 |
| A | WILES M V ET AL: "Establishment of a gene-trap sequence tag library to generate mutant mice from embryonic stem cells" NATURE GENETICS, NATURE AMERICA, NEW YORK, US, vol. 24, January 2000 (2000-01), pages 13-14, XP002180070 ISSN: 1061-4036 the whole document | 1-29 |
| A | WO 99 29837 A (EUROP LAB MOLEKULARBIOLOG ;ZHANG YOUMING (DE); BUCHHOLZ FRANK (DE)) 17 June 1999 (1999-06-17) the whole document | 1-29 |
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INTERNATIONAL SEARCH REPORT

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PCT/CA 02/01160

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
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| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| P,X | WO 01 60975 A (BOONE CHARLES ;BUSSEY HOWARD (CA); JIANG BO (CA); ROEMER TERRY (CA) 23 August 2001 (2001-08-23) abstract page 10, line 7 - line 20 page 16, line 10 -page 18, line 14 page 23, line 7 -page 26, line 14 page 32, line 28 -page 35, line 8; table II page 93, line 1 -page 95, line 10 page 109, line 9 -page 110, line 34; figure 2 ----- | 1-29 |

INTERNATIONAL SEARCH REPORT

international application No.
PCT/CA 02/01160

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-29 (partially), 30 (completely)
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-29 encompass methods that involve an invasive step i.e. the provision of a host cell, the search has been carried out and based on methods carried out on isolated cells.

Continuation of Box I.1

Claims Nos.: 1-29 (partially), 30 (completely)

Rule 39.1(v) PCT - Presentation of information Rule 39.1(iv) PCT
-Claims 1-29 encompass methods that involve an in vivo step carried out on a human or animal body, namely the provision of a host cell of an organism (step (a)).

Rule 39.1(v) PCT - Presentation of information - Claim 30 is directed to a database, which is mere presentation of information completely lacking technical features

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 02/01160

| Patent document cited in search report | | Publication date | Patent family member(s) | Publication date |
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